510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k041173

B. Purpose for Submission:
New device

C. Analyte:
Anti-tissue transglutaminase (tTG) IgG antibody

D. Type of Test:
Qualitative and semi-quantitative, EIA

E. Applicant:
Sweden Diagnostics (Germany) GmbH
Pharmacia Diagnostics AB

F. Proprietary and Established Names:
Celikey® Tissue Transglutaminase (human, recombinant) IgG Antibody

G. Regulatory Information:

1. Regulation section:
   21 CFR §866.5660, Multiple Autoantibodies Immunological Test System

2. Classification:
   Class II

3. Product Code:
   MVM, Autoantibodies, endomysial (tissue transglutaminase)

4. Panel:
   Immunology (82)

H. Intended Use:

1. Intended use:
   Celikey® tTG (human, recombinant) IgG Antibody Assay is intended for the semiquantitative and qualitative measurement of anti-tissue transglutaminase (tTG) IgA antibodies in human serum and plasma. Celikey is based on recombinant human tissue transglutaminase as antigen and is useful as an aid in the clinical diagnosis of patients with celiac disease.

2. Indication(s) for use:
   Same as Intended Use.

3. Special condition for use statement(s):
   The device is for prescription use only.

4. Special instrument Requirements:
   Microplate reader capable of measuring OD at 450 nm and with a reference filter at 620 nm.

I. Device Description:

The assay kit consists of (1) 12 or 6 human recombinant tTG coated microplate strips, (2) horseradish peroxidase conjugated anti-human IgG, (3) TMB substrate, (4) ready-to-use 6 level calibrators (tTG antibody concentrations of 0, 3, 7, 16, 40 and 100 U/mL), (5) ready-to-use positive control, (6) ready-to-use negative control, (7) wash buffer concentrate (20x), (8) sample diluent concentrate (5x) and (9) stop solution. Calibrators, positive and negative controls are diluted human sera.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   INOVA QUANTA Lite™ h-tTG (human tissue transglutaminase)

2. Predicate K number(s):
   k011570

3. Comparison with predicate:

DEVICE	PREDICATE
A. Similarities	
Intended Use. For the semiquantitative and qualitative measurement of anti-tissue transglutaminase (tTG) IgG antibodies in human serum and plasma. Celikey is based on recombinant human tissue transglutaminase as antigen and is useful as an aid in the clinical diagnosis of patients with celiac disease.	For the semi-quantitative detection of IgG antibodies to tissue transglutaminase (endomysium) in human serum. Detection of these antibodies in conjunction with IgA antibodies, is an aid in diagnosis of certain gluten sensitive enteropathies such as celiac disease and dermatitis herpetiformis. This test is intended for providing added sensitivity when testing IgA deficient patients.
Assay type – ELISA	Same
Analyte – Anti-tTG IgG antibody	Same
Capture antigens – tissue transglutaminase	Same
Conjugate - Horseradish peroxidase	Same
Substrate – TMB	Same
Sample dilution – 1:101	Same
B. Differences	
Assay format – Qualitative and semi-quantitative	Semi-quantitative
Source of tTG – human recombinant antigen from baculovirus/insect cell system	Native human antigen isolated from RBC
Sample type – Serum and plasma	Serum
Calibrators – 6 levels	None

DEVICE	PREDICATE
A. Similarities	
Controls – positive and negative	Negative, low positive and high positive controls
Cut-off values	
Semi-quantitative - negative <7 U/mL equivocal 7-10 U/mL positive >10 U/mL	Negative <20 units weak positive 20-30 units moderate to strong positive >30 units
Qualitative - negative ratio <1.0 equivocal ratio 1.0-1.4 positive ratio >1.4	

K. Standard/Guidance Document Referenced (if applicable):

None referenced.

L. Test Principle:

The Celikey tTG IgG Antibody assay is an indirect noncompetitive enzyme immunoassays. The wells of a microplate are coated with recombinant human tTG antigen. Diluted patient samples are added to the microplate wells and antibodies specific for tTG if present will bind to the immobilized antigen. Unbound samples are washed away and an enzyme labeled second antibody (conjugate) is added to each well and bind to the antigen/antibody complex to form an enzyme labeled conjugate-antibody-antigen complex. After washing away any unbound enzyme conjugate, the chromogenic substrate is added. The enzyme labeled antigen-antibody complex converts the substrate to form a color solution. The rate of color formation is a function of the amount of conjugate complexed with the bound antibody and therefore is proportional to the concentration of the autoantibody in the patient sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Three QC samples (low, medium, high) from a serum bank were diluted and assayed for 5 runs with 4 replicates per run. Calibrators and Controls were analyzed in triplicates. Target values established for this study included within-run variance <12% and between assay <8%. The specifications were met. The table below summarizes the results.

Sample ID		Run 1	Run 2	Run 3	Run 4	Run 5	Mean (U/mL)	Variance	
								Within	Between
QC3 (1:150)	Mean (U/mL)	10.6	9.8	9.0	9.4	9.6	9.6	6.6	4.9
	CV%	5.66	5.83	6.85	4.98	8.91			
QC4 (1:150)	Mean (U/mL)	20.2	19.6	19.4	19.6	20.4	19.8	3.6	1.5
	CV%	3.27	2.56	5.41	4.43	0.73			
QC5 (1:400)	Mean (U/mL)	31.6	32.5	33.3	36.6	35.4	33.8	7.2	4.5
	CV%	8.97	6.34	9.37	5.76	4.93			

b. Linearity/assay reportable range:

Dilution study - Four positive serum samples from a serum bank and Calibrator S6 were used in this study. Depending on the tTG IgG antibody concentration, each sample was pre-diluted to a specified dilution prior to further dilution with Sample Diluent to 1:1, 2:3, 1:2, 1:4, 1:8, 1:16, and 1:32. Same dilutions were made to Calibrator S6. Calibrators, Controls and each dilution were measured in duplicates. Specifications were that observed/expected percents should be within ± 20% for at least 3 successive dilutions of each tested sample. The dilutions met the criteria and were considered linear.

Recovery study – Two samples containing 29.4 U/mL and 58.4 U/mL of tTG IgG selected from a serum bank were diluted to 1:101 and spiked with 1/10 volume of Calibrator points S1, S2, S3, S4, S5 and S6, i.e. 0.3, 0.7, 1.6, 4 and 10 U/mL. The unspiked and spiked samples, the Calibrators and controls were measured in duplicates. Acceptance criteria were that recovery (%) ± 20% of the expected values. The lower concentration sample had percent recoveries ranged from 89.5% to 95.2% and the high concentration sample from 89.3% to 100%. Study results met the acceptance criteria.

Reportable range – 0.5 U/mL to 100 U/mL

c. Traceability (controls, calibrators, or method):

There is no recognized reference material for tTG autoantibodies. Results are reported in arbitrary units.

d. Detection limit (functional sensitivity):

Sample Diluent was diluted according to Directions for Use and measured 56 times on one plate. Calibrators and Controls were analyzed in quadruplicates. Analytical sensitivity was calculated as the mean of the optical densities (OD) of the Sample Diluent plus 3SD and expressed in U/mL. The discrimination value D for differentiating the lowest calibrator point and the background was calculated using the following equation:

$$D = \frac{\eta_B - \eta_A}{\sqrt{(\sigma_B^2 - \sigma_A^2)}}$$

where A = Sample Buffer; B = Calibrator S2; $\acute{\eta}_A$, $\acute{\eta}_B$ = mean OD; σ_A, σ_B = SD.

Acceptance criteria specified that the (mean OD + 3SD) of the Sample Diluent < Calibrator point S2, detection limit ≤1 U/mL and the discrimination value D >2.0. The (mean OD +3SD) was 0.027

for the Sample Diluent, which corresponded to analytical sensitivity of <0.1 U/mL. The other criteria were also met. The lower limit of the measuring range was set to 0.5 U/mL.

e. *Analytical specificity:*
Interference was tested against potentially interfering substances found in blood: bilirubin, hemoglobin, chyle, and rheumatoid factor. Three samples with known tTG IgG concentration from a serum bank were diluted 1:101 and spiked with buffer or different amounts of interfering substances. The spiked and unspiked samples were analyzed in triplicates. The Calibrators and Controls were analyzed in duplicates. Acceptance criteria were that spiked samples should be ≤20% variation from unspiked sample. The concentrations of the spike-in substances are shown in table below.

	Final sample concentration				
Additives	Blank	II	III	IV	V
Bilirubin F (mg/dL)	0	4.7	9.4	14.1	18.8
Bilirubin C (mg/dL)	0	5	10	15	20
Chyle (Units)	0	590	1180	1770	2360
Hemoglobin (mg/dL)	0	113.3	226.5	339.8	453
RF (IU/mL)	0	106	318	530	n.a.-

All samples met acceptance criteria and showed no significant interference on the test results (see below).

	Mean	Recovery (%)			
Additives	(U/mL)	II	III	IV	V
Bilirubin C	57.5	108.5	104.9	96.2	105.6
	53.6	103.4	104.4	98.0	100
	47.5	109.5	106.7	99.2	104.0
Bilirubin F	60.2	102.9	99.3	97.5	102.5
	51.8	100.8	104.4	107.6	104.1
	50.7	102.3	99.3	96.5	102.8
Chyle	58.3	104.6	96.3	98.2	99.8
	54.3	101.5	95.2	96.9	97.4
	51.4	94.7	91.2	90.8	91.8
Hemoglobin	57.8	100.8	100.1	95.5	100.6
	53.0	98.8	101.6	98.1	102.2
	48.2	98.9	100.1	99.2	105.7
RF	57.7	107.9	108	105.4	n.a.
	64.9	102.9	102.2	104.4	n.a.
	46.1	100.7	108	111.1	n.a.

Two additional samples (1 negative and 1 equivocal) were added to the interference study because the original samples had high tTG IgG concentrations and might not be affected by the amount of interfering substances spiked-in. Results showed no significant interference was observed.

Crossreactivity was assessed by testing 60 sera positive for other antibodies including ANA, SS-A, SS-B, TPO, dsDNA Cardiolipin IgG, PR3, GBM, MPO, RF, PC, LKM1, actin and HCV. Fifty sera were from an external source and 10 sera were ANA human reference sera from CDC. All samples were found negative.

f. Assay cut-off:

The semi-quantitative cut-points were determined by measuring 432 samples from apparently healthy blood donors, equally distributed by sex and age. The samples were from the serum bank at the company. Diluted samples, Calibrators and Controls were analyzed in duplicates. Specification was that 95th percentile should be <lower limit of equivocal range. Results showed that there was no difference between gender and age. The mean and median concentration of tTG IgG antibodies were 0.9 U/mL and 0.7 U/mL respectively. The mean+2SD was 2.5 U/mL, the mean+3SD was 3.3 U/mL and the 95 percentile was 2.1 U/mL. Based on these results, the following values were selected for negative, equivocal, and positive:

<7 U/mL = negative
7-10 U/mL = equivocal
>10 U/mL = positive

2. Comparison studies:

a. Method comparison with predicate device:

One hundred and fifteen clinically defined patient samples and forty-two normal control samples from a serum bank were tested on the new device, the predicate device and an IIF anti-endomysial (EMA) assay. The patient samples consisted of 70 celiac disease (CD), 19 inflammatory bowel disease (IBD) and 16 Morbus Crohn/Colitis Ulcerosa (Crohn/UC). The concentration ranges of anti-gliadin IgG antibody for the four cohorts are shown in the following table.

Celikey® tTG IgG (U/mL)	Cohorts			
	CD	Crohn/UC	IBD	Healthy
N	80	16	19	42
Range	1.2 to >100	0.6 to 4.1	3.2 to 6.7	0.3 to 3.3

		INOVA QUANTA Lite h-tTG IgG		
		+	-	Total
Celikey tTG IgG	+	13	33	46
	-	1	103	104
	Equiv	0	7	7
	Total	14	143	157

% positive agreement = 92.9% (13/14)
% negative agreement = 72.0% (103/143) (95% CI 64.6% to 79.4%)

% total agreement = 73.9% (116/157), (95% CI 67.0% to 80.8%)

The following table is a compilation of the discrepant samples.

Sample Type	INOVA^{Pos}/Celikey^{Neg}	INOVA^{Neg}/Celikey^{Pos}	INOVA^{Neg}/Celikey^{Equ}
CD	0	33	7
Crohn/UC	0	0	0
IBD	0	0	0
Healthy	1	0	0
Total	1	33	7

The discrepancy between the assays could most likely be due to the source of tTG antigen used for capture. The Celikey tTG antigen is a recombinant human antigen produced by a baculovirus/insect cell system whereas the Quantia Lite tTG antigen is a native antigen isolated from human red blood cells.

b. Matrix comparison:

The device uses both serum and plasma samples. To demonstrate that the new assay gives the same results for serum, heparin plasma, citrate plasma and EDTA plasma from the same patient, 10 tTG IgG antibody negative samples (tTG IgG concentrations ranged from 1.6 U/mL to 3.3 U/mL) for each matrix were spiked with 10 different tTG IgG positive sera (tTG IgG concentrations ranged from 9.4 U/mL to 78.7 U/mL). The negative samples, the calibrators and controls were run in duplicate. The spiked samples were run in quadruplicate. Acceptance criteria for this study were that the percent deviation between serum and plasma results for positive samples should not be greater than ± 20% and negative samples should be negative for both serum and plasma matrices. The data showed no difference greater than ± 20% with deviations ranging from -5.9% to 15.7% for citrate, -10.9% to 16.7% for EDTA plasma and 4.1% to 17.5% for heparin. No negative sample changed from negative to positive. Thus the specifications were met.

The matrix comparison study was expanded using serum and plasma samples from two additional patients (one negative with 4.9 U/mL and one equivocal with 8.8 U/mL). Results were within the acceptance criteria.

3. Clinical studies:

a. Clinical sensitivity:

One hundred and sixty two clinically defined sera were analyzed using the Celikey® tTG IgG Antibody Assay and the IFA EMA assay. The samples consisted of 83 CD, 19 IBD, 17 Crohn/UC and 43 normal controls. In the CD cohort, eight subjects had equivocal tTG IgG results which were EMA positive. In the normal cohort,

there was one Celikey equivocal sample and this sample was EMA negative. Samples with equivocal results were considered negative in calculation of sensitivity and specificity. Based on this study, the clinical sensitivity of the Celikey tTG IgG assay was 55.4% (95% CI 44.7% to 66.1%). Results are summarized below:

		Celiac disease		
		Positive	Negative	Total
Celikey IgG	Positive	46	0	46
	Equivocal	8	1	9
	Negative	29	78	107
	Total	83	79	162

b. Clinical specificity:
The clinical specificity of the Celikey tTG IgG assay was determined by the study described in 3(a). The normal cohort had one equivocal sample that was EMA negative. Since the sponsor considered equivocal samples negative, clinical specificity of the Celikey tTG IgG was 100%.

4. Clinical cut-off:
Same as assay cut-off.

5. Expected values/Reference range:
Expected value in the normal population is negative. The frequency distribution for tTG IgG antibodies as determined by the Celikey® tTG IgG Antibodies assay on the 162 clinically defined samples is summarized below.

	N	#Positive	Frequency
CD	83	46	55.4%
IBD	19	0	0%
Crohn/UC	16	0	0%
Healthy	43	0	0%

N. Conclusion:
The submitted information in this premarket notification is complete to support a substantial equivalence decision.